

# Morphological analysis of phytoplankton as a tool to assess ecological state of aquatic ecosystems: the case of Lake Arancio, Sicily, Italy

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Received 30 August 2013; accepted 25 October 2013; published 25 November 2013

## Abstract

Phytoplankton requires a sufficient supply of light and nutrients to grow. At the same time it is largely entrained in water motion and is subject to grazing from planktonic herbivores. The pelagic life of lake phytoplankton is based on 4 pillars: living in suspension, light harvesting, nutrient uptake, and escape from grazing. Environmental variability and the uneven distribution of resources among and within the different aquatic ecosystems exert a selective pressure on these organisms, which are formed by a single cell or by colonies with either relatively low or high numbers of cells. Phytoplankton displays an amazing morphological variability representing an adaptation to spatial and temporal variability of their environments. Among functional traits of phytoplankton, morphological features such as shape and size represent an unavoidable starting point to fully understand the relationships between this group of organisms and its environment. The molding effect of environmental variables on phytoplankton morphology is particularly evident in highly dynamic environments such as reservoirs or ponds. The analysis of phytoplankton morphology can thus supply important information on the ecological conditions of a given waterbody. To support this hypothesis, data are presented on the main morphological features of phytoplankton from a 20-year limnological investigation carried out in a Mediterranean man-made lake. The results show that phytoplankton morphological variability reflects different environmental scenarios, and that the morphological features of phytoplankton represent a powerful tool to assess the ecological or trophic state of aquatic ecosystems.

**Key words:** C-S-R strategies, freshwater phytoplankton, functional groups, maximal linear dimension, shape, size, surface, volume

## Introduction

The variety of shapes and sizes of “visible” organisms has fascinated humans since the beginning of their history on Earth. Through the distinction of different shapes and sizes, early human beings began classifying organisms into categories. The importance of naming, thus recognizing different organisms, had strong survivorship implications for a society based on food collection and hunting and is even acknowledged in the first pages of the Bible (Genesis, 2, 19–20). In the last century, the study of relationships between size and shape from an adaptive and

evolutionary perspective was a major scientific interest of paleontologist Stephan Jay Gould, who dedicated several scientific papers to this topic (e.g., Gould 1966, 1968, Alberch et al. 1979) and stimulated more general attention through a number of famous essays published in his “This View of Life” column in *Natural History* (e.g., Gould 1974). Among aquatic ecologists, the modern debate on the physiological and ecological implications of body size finds its roots in the elegant writings of Robert Henry Peters (1983), whose publications renewed the interest of marine and freshwater biologists in issues related to body size (Hildrew et al. 2007).

The invention and enhancement of microscopes in the 17<sup>th</sup> century led to the discovery of an “invisible” world of organisms also showing a strong variability in shape and size, although these organisms were mainly formed by a single cell. A starting point to clarify the biological meaning of this variability can be found in the book “On Growth and Form” by D’Arcy W. Thompson (1917, reprinted in 1942). This author first realized that physical laws acting on “visible organisms” are different from those governing the life of “invisible organisms.” He faced the problem of scale and separated those organisms with physical forces acting either directly at the body surface, or otherwise in proportion to their surface, from those with forces (above all gravity) acting on all particles, resulting in a force proportional to the mass or volume of the body. In synthesis, he pointed out the need to distinguish among the forces acting on organisms living at low and high Reynolds numbers and opened the way to a series of studies on the adaptive value of size and shape among unicellular organisms. Among these is a book by Edward F. Adolph, *The Regulation of Size as Illustrated in Unicellular organisms* (Adolph 1931), in which he collected almost all available knowledge at the beginning of the last century on the implication of cell size on the metabolism of unicellular organisms. Since then, this topic has steadily attracted the attention of biologists, as reviewed by Naselli-Flores and Barone (2011).

Among unicellular organisms, phytoplankton has a prominent role. Although it accounts for <1% of the photosynthetic biomass on Earth, phytoplankton production is nearly 50% of the global net primary production (Salmaso et al. 2012). Phytoplankton is an ecological group of photosynthetic, unicellular, or colonial organisms adapted to live in suspension in open waters. Its members can be found in almost all surface aquatic ecosystems under a wide variety of environmental conditions, and thus may be considered one of the most important engines fueling the functioning of the biosphere. Moreover, phytoplankton offers an amazing morphological variability in both size and shape at an intra- and inter-specific level (Naselli-Flores et al. 2007); in particular, length variability spans 4 orders of magnitude and body mass 7 orders of magnitude. From a human perspective, the size difference between small and large phytoplankton is similar to that between a poppy and an oak; no one would say that these 2 organisms share common ecological features.

Similarly, the shape of phytoplankton, both single-celled and colonial species, is extremely variable. These features have been traditionally used only for taxonomic classification of organisms. Reynolds (1997, 2006, and literature therein) explained in detail in 2 seminal books summarizing a life-long work how the diverse ecological

strategies adopted by phytoplankton can be related to differences in their morphologies, which thus represent a fundamental adaptive value (Naselli-Flores and Barone 2011); the environmental differences among waterbodies, acting as a sieve, select the best-fitting morphologies.

Living in suspension in open waters and entrainment in water motion is key to understanding vegetation processes in the pelagic. Nutrient uptake and light harvesting performances can be related to morphological features of phytoplankton and are mediated by their sinking and/or floating velocity in relation to the mixing regime of a given waterbody. For organisms living at low Reynolds numbers ( $\ll 1$ ), sinking–floating velocity can be calculated by the Stoke’s equation, originally intended for spherical objects and later modified to include a species-specific coefficient of form resistance that considers the impact of the deviation from the spherical shape on the sinking behaviour:

$$v' = 2/9 \, g r^2 (\rho' - \rho) \eta^{-1} \varphi_r^{-1} \, [\text{m s}^{-1}], \quad (1)$$

where  $v'$  [ $\text{m s}^{-1}$ ] is the sedimentation velocity of the sphere;  $g$  [ $\text{m s}^{-2}$ ] is the acceleration of gravity;  $r$  is the radius of the sphere [ $\text{m}$ ];  $\rho'$  [ $\text{kg m}^{-3}$ ] is the density of the sinking sphere;  $\rho$  [ $\text{kg m}^{-3}$ ] is the density of the fluid;  $\eta$  [ $\text{kg m}^{-1}\text{s}^{-1}$ ] is the viscosity of the fluid; and  $\varphi_r$  is the coefficient of form resistance, which can be measured experimentally (Padisák et al. 2003) and expresses the factor by which the sinking velocity of the particle differs from that of a sphere of identical volume and density in the same liquid. If  $\varphi_r$  is  $>1$ , the associated shape will sink slower than the equivalent sphere; if it is  $<1$ , the shape will sink faster than that of the equivalent sphere (for a more detailed account see Naselli-Flores and Barone 2011).

Three adaptive features are included in the Stoke’s equation. Two depend strictly on size (the radius of the equivalent sphere) and shape (the coefficient of form resistance), and the third,  $\rho' - \rho$ , also called excess of density, determines whether a cell is sinking ( $\rho' > \rho$ ) or floating ( $\rho' < \rho$ ), dependent on functional, adaptive features of the organisms other than size or shape (e.g., presence of vacuoles or gas vesicles, type of storage substances, typology of cell wall).

Phytoplankton morphological diversity can therefore be considered a key factor allowing this ecological group to cope with the various environmental conditions experienced in aquatic ecosystems. By understanding the adaptive value of different phytoplankton morphologies, the ecological state of aquatic ecosystems can be assessed. To support this idea, a morphological analysis of phytoplankton was conducted in a highly dynamic, man-made Mediterranean lake over 16 years.

## Material and methods

### Study site

Lake Arancio is a eutrophic–hypereutrophic man-made lake in southwestern Sicily (Italy), 85 km south of Palermo and 5 km from Sicily's southern coast. In past years, the high trophic state of the lake has produced heavy blooms of *Microcystis* spp., causing concern in the Management Board. The reservoir, which is mainly used to supply water for agriculture purposes during the irrigation period (Jun–Oct), receives untreated urban wastes from a small town with 7000 inhabitants located 1 km from its shores. A wine factory and a swine farm located directly on its shores also contribute to nutrient loads. The trophic state of the lake was estimated as one of the most eutrophic among 21 Sicilian reservoirs on the basis of samples collected seasonally in 1987 (Naselli-Flores and Barone 1994), prompting a limnological investigation of the ecosystem that began at the end of 1989 and ended in 2009. A series of publications resulting from these studies (Barone and Naselli-Flores 1994, Naselli-Flores 2003, Naselli-Flores and Barone 1997, 1998, 2000, 2003, 2005, 2007) detail all analytical methods as well as the instruments used in the present study. The previous studies clarified the role of water-level fluctuations on the physical and chemical characteristics of the lake and, ultimately, on phytoplankton, particularly *Microcystis* blooms.

Lake Arancio, based on morphology, is a warm monomictic lake, with stratification usually beginning the end of March and circulation occurring in mid-November (Naselli-Flores 2003); however, the agricultural water demand can quickly reduce its depth and disrupt the thermocline in midsummer. The region is characterized by a semi-arid Mediterranean climate, and water inflow to the reservoir is confined to the winter months. During summer there is no inflow to compensate for the strong drawdown caused by evaporation and by water usage to satisfy agricultural requirements. Consequently, the waterbody experiences strong water-level fluctuations on an annual basis, on which a longer (11–12 years) mesoclimatic periodicity is superimposed (Mosetti 1996, Padisák 1998). Due to typical Mediterranean climatic oscillations and variation in the amount of precipitation, this periodicity causes high and low water-level years. The main morphological features of the waterbody thus vary from season to season and year to year; as a consequence, no macrophytes inhabit the waterbody, and primary production is largely due to phytoplankton.

The lake has a theoretical maximum water level at 180 m a.s.l., which corresponds to a stored volume of  $32 \times 10^6 \text{ m}^3$ , a water surface of 3.2 km<sup>2</sup>, a maximum depth

of 30 m, and an average depth of 10 m, but these values have seldom been attained since the dam was built. To compensate for low water inflows and to accumulate as much water as possible, a complicated feeding system was implemented in which the reservoir receives water both from its own catchment area of about 140 km<sup>2</sup> and from 3 neighbouring catchments (about 65 km<sup>2</sup>) connected through pipelines, which irregularly supply water to the lake. An additional water pipeline connects another reservoir, Lake Garcia, to Lake Arancio. Lake Garcia is a mesotrophic, relatively deep (43 m) reservoir, located about 30 km North of Lake Arancio (Naselli-Flores and Barone 2005). It reached pool level in 1992 to supply water to the city of Palermo, but it has never been used for this purpose because the distribution network has not been completed. Since 1998 it has been regularly used as an additional source of water to feed Lake Arancio, providing  $13 \times 10^6 \text{ m}^3 \text{ y}^{-1}$ .

To better understand the effects caused by water level fluctuations, in 2003 a proposal to modify the strategy for refilling Lake Arancio was made to the Management Board, who agreed to gradually refill the reservoir as the water was used rather than adding water from Lake Garcia all at once when the volume of Lake Arancio reaches the minimum. This modified hydraulic input was chosen to minimize thermocline disturbance in the summer period, maintain the thermal stability of the reservoir, favor epilimnetic nutrient consumption by phytoplankton, and lessen nutrient pulses from sediments during summer (Naselli-Flores and Barone 2005).

### Data collection

Phytoplankton samples were collected at a fixed station located 200 m off the central part of the dam at discrete depths within the euphotic zone using a 5 L Van Dorn bottle. The data presented here, including measures of individual volume and surface, were collected weekly in 2 periods (1990–1993 and 2000–2004) and monthly in the remaining periods (1994–1999 and 2005); phytoplankton data represent 1990–2005. Phytoplankton counts were performed according to the Utermöhl method (Lund et al. 1958), and measurements were taken under a Zeiss Axiovert 100 inverted microscope equipped with an Axio-Cam camera, using the Zeiss software AxioVision. Average surface, volume, and maximal linear dimension of the taxa were calculated or recalculated according to Hillebrand et al. (1999). For colony-forming organisms, the shape and measurements of the colony were considered. Several hundred measurements were taken during the investigation period, allowing an estimate of average biovolume of single species and the total biovolume, which were used as a proxy of biomass.

To unify the dataset and simplify interpretations, weekly data on phytoplankton were pooled into monthly averages and a single taxon, characterized by the highest average biomass value identified as “the dominant” of that month.

Two morphological descriptors of the phytoplankton taxa were plotted on an X–Y graph month by month. The descriptors were a dimensionless shape descriptor calculated from the product of maximal linear dimension and the surface–volume ratio ( $m\ sv^{-1}$ ) along the X axis, and a size descriptor (in  $\mu m^{-1}$ ) given by the surface–volume ratio ( $sv^{-1}$ ) along the Y axis. This method distributes the taxa inside a triangle with corners representing the 3 main theoretical strategies (C–S–R) adopted by phytoplankton (Reynolds 1997). Moreover, as reviewed in detail by Reynolds (2006 and literature therein), several studies emphasized the influential nature of the relationship between algal shape in the interception of light energy and the impact of algal size in governing metabolism. These 2 morphological descriptors are thus good proxies for light availability ( $m\ sv^{-1}$ ) and nutrient availability ( $sv^{-1}$ ). The biomass values of those species falling within the range of a single survival strategy were then grouped together. All identified species were included within one of the 3 strategies; the ecological characteristics of these survival strategies are described in Reynolds (2006, Box 5.1).

In addition, phytoplankton taxa were also grouped into coda (functional groups) according to Padisák et al. (2009); species not listed in that study were sorted into coda according to the methodology described in Reynolds et al. (2002).

Temperature and irradiance profiles, as well as samples for nutrients, were taken with the same frequency as phytoplankton sampling. Chlorophyll *a* measures were conducted in 1990–1993 and 2001–2002. Samples for nutrient and chlorophyll *a* analyses were collected concurrently with phytoplankton samples; analytical methods were reported in Barone and Naselli-Flores (1994) and Naselli-Flores and Barone (2007). Nutrients are not limiting phytoplankton growth in the hypereutrophic Lake Arancio, and species selection is mainly driven by light conditions (Naselli-Flores and Barone 1998). The synthetic descriptor  $z_{mix}/z_{eu}$ , a proxy of underwater light availability, was identified as the driving variable modulating phytoplankton dynamics in this environment (Naselli-Flores 2000, Naselli-Flores and Barone 2007). The ratio  $z_{mix}/z_{eu}$  is an extremely useful descriptor to estimate the underwater light climate of a given waterbody because it indicates the proportion of time an alga has to spend in poor light conditions once it is fully entrained in the mixed water column. Methods

used for evaluating  $z_{mix}$  and  $z_{eu}$  were described in detail in Naselli-Flores and Barone (2003).

Data on water levels were supplied by the Management Board of the reservoir. These values are collected daily and allow calculation of stored volumes through a conversion equation, which is regularly updated to consider sedimentation.

## Results

### Hydrological and thermal patterns in the study period

The lake exhibited large water level fluctuations during the study period (Fig. 1), both intra- and inter-annually. In particular, the first 3 years of investigation were characterized by low levels and by the absence of a stable stratification. In 1992, the lake stratified for a brief period (Fig. 2), but the water abstraction at the beginning of the irrigation period reduced the stored volume and caused the breakdown of the thermocline in June. Due to increased precipitation, 1993 was characterized by higher stored volumes, and the lake had a prolonged stratification period that lasted until the end of July. The stored volumes were also high during 1994–1997 with a peak in 1995, and this allowed the thermocline to persist throughout summer. Again, 1998–2002 was characterized by a break in the thermocline due to the high and increased withdrawals of water for irrigation. Since 1998, the Management Board of the Lake Arancio dam has conveyed water from Lake Garcia to fulfill the increased water demand for irrigation. The water was added to the reservoir at the end of summer (late Sep–Oct) when the retained volumes in Lake Arancio were too low to continue abstracting water to satisfy the high agricultural demands. From 2003 onward, high water levels (and stored volumes) again occurred in Lake Arancio, also supported by the new refilling strategy (see study site section). Since 2003, a dewatering threshold was established at  $12.5 \times 10^6\ m^3$  to avoid the anticipated break in the thermocline. Analysis of the entire study period detected 3 main hydrological/thermal patterns in the lake:

**A periods:** very low water levels. The lake did not store enough water to exhibit summer stratification.

**B periods:** intermediate water levels. The lake had sufficient water in spring for stratification to occur, but water abstraction caused a break in the thermocline in midsummer.

**C periods:** high water levels. The lake stored sufficient water to exhibit a warm monomictic behavior with a stable thermocline throughout summer.



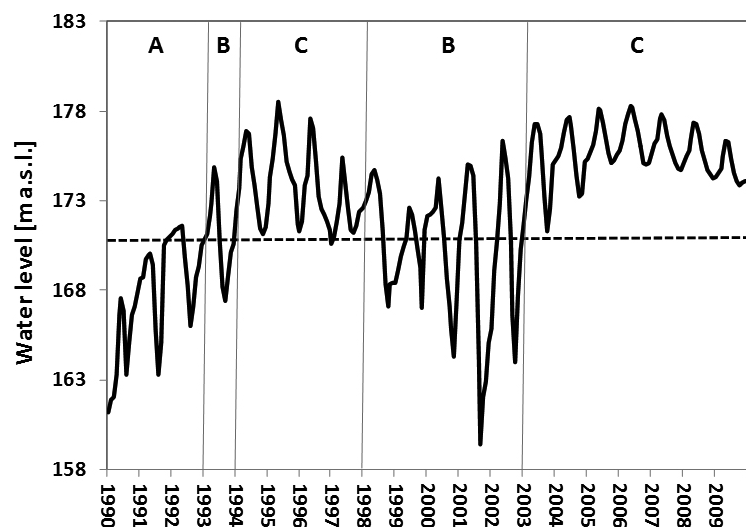


Fig. 1. Inter- and intra-annual variability of water stored in Lake Arancio, Italy, during the study period. The horizontal line indicates the dewatering threshold, above which the waterbody maintains its summer stratification. A, B, and C represent 3 periods when the lake could be considered as shallow (A), deep (C) or intermediate (B).

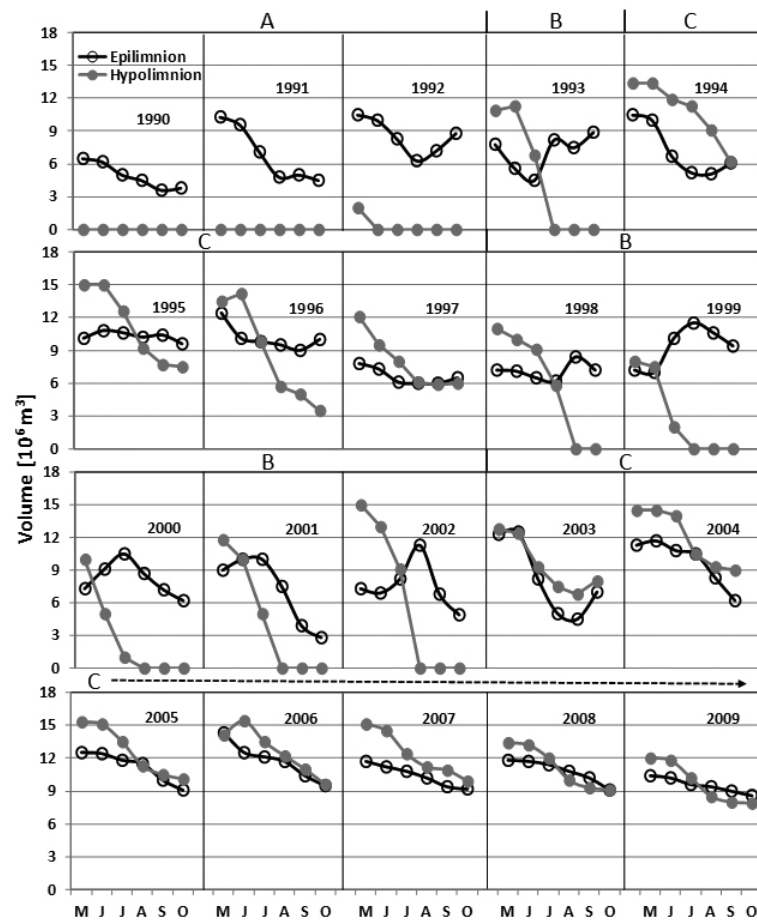


Fig. 2. Trends of epilimnetic and hypolimnetic volumes in Lake Arancio, Italy, during the stratification period.

## Underwater light climate fluctuations

During the study period the hydrological and thermal characteristics of the lake influenced its light regime (Fig. 3). The ratio between mixing depth and euphotic depth generally oscillated between 1.0 and 3.5 during the periods A and C, when the lake was similar to a shallow (absence of summer stratification) and to a deep lake (stable summer stratification), respectively. Conversely, when a break in the thermocline occurred (period B) much higher ratio values were recorded. In particular, the 5 years between 1998 and 2002 were characterized by a progressively decreasing availability of light in the mixing zone during summer and autumn, and the second part of 2002 showed  $z_{\text{mix}}/z_{\text{eu}}$  values  $>7$ , thus indicating very low light availability in the mixing zone.

## Nutrient availability

Two main sources of nutrients reaching Lake Arancio were recognized: an external source derived from intense anthropogenic activities in the watershed, and an internal source connected to the hydrological and thermal regime experienced by this ecosystem. The internal source was clearly evident by comparing weekly values of soluble reactive phosphorus (SRP) measured in two 4-year periods (Fig. 4). In the first period (1990–1993), values fluctuated around  $20 \mu\text{g P L}^{-1}$  (Fig. 4a), but summer SRP values had peaks close to  $100 \mu\text{g P L}^{-1}$  in 1990. During summer, all water sources feeding the reservoir were dry, and thus these phosphorus pulses were from the internal loading of the reservoir, likely promoted by the lowering thermocline caused by dewatering or by episodes of atelomixis. This proposed process was also supported by contemporary pulses of  $\text{N-NH}_4$  (data not shown). During this period of internal load, SRP concentrations showed an increasing trend, although not significant. In 2001–2004 the summer peaks, although still present, were less evident when compared to the values measured in the rest of the year (Fig. 4b). Conversely, the inputs from the watershed coinciding with the Mediterranean rainy season (mid-autumn–early spring), increased compared to 1990–1993,

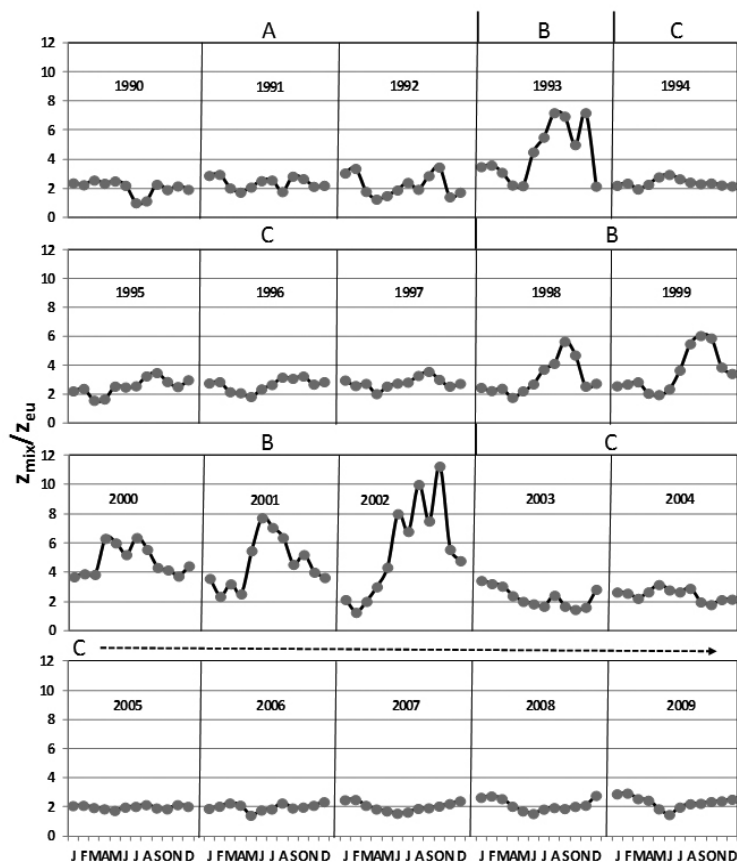


Fig. 3. Annual variations of  $z_{\text{mix}}/z_{\text{eu}}$  in Lake Arancio, Italy, during the study period.

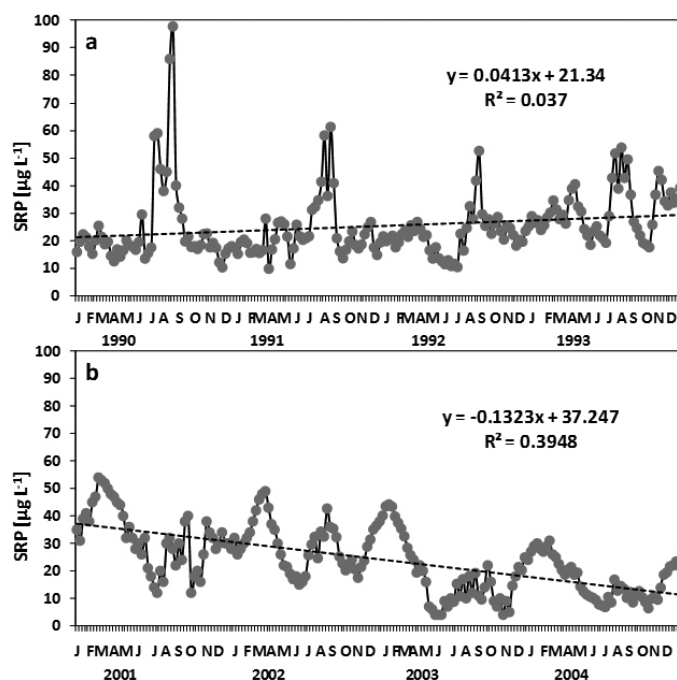


Fig. 4. Variability of solid reactive phosphorus (SRP) concentrations in Lake Arancio, Italy, during (a) 1990–1993 and (b) 2001–2004. Dashed lines represent the trends in the periods.

suggesting a relatively higher contribution from the external load; however, a significant trend toward lower SRP concentrations was observed.

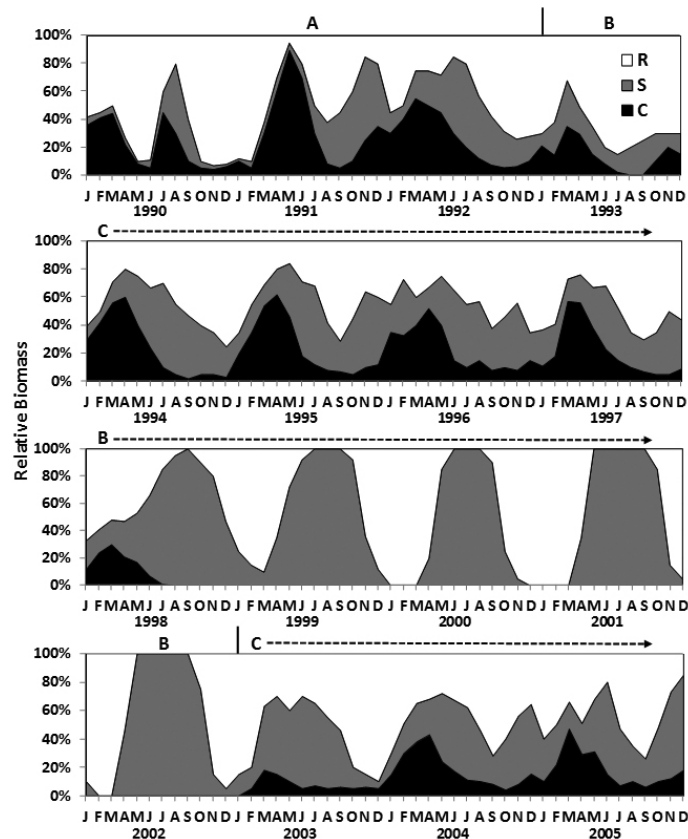
### Morphology-based phytoplankton trends

The temporal development of the main phytoplankton strategies, established on a strict morphological basis, was in close agreement with underwater light availability as described by the  $z_{\text{mix}}/z_{\text{eu}}$  ratio (Fig. 5). In particular, C-strategists developed in winter and early spring and were followed by S-strategists in summer and by R-strategists in autumn. This pattern was regular during the study period, except in the years characterized by breaks in the thermocline and by  $z_{\text{mix}}/z_{\text{eu}} > 3.5$  (B periods in Fig. 1). In these periods, the share of C-strategists decreased (1993) or completely disappeared during the summer break in the thermocline over several consecutive years (1998–2002). In those years, an absolute dominance of S-strategists characterize Lake Arancio from April to November, alternating with R-strategists from December to March. Since establishing a dewatering threshold (2003) to maintain the thermocline throughout summer, the C→S→R sequence of survival strategies has again been observed in the lake.

A more detailed analysis of phytoplankton performed by grouping the species into functional groups revealed a change in their composition (Table 1). In particular, during years when the reservoir was “shallow” (A periods in Fig. 1) or “deep” (C periods in Fig. 1), with  $z_{\text{mix}}/z_{\text{eu}}$  values  $< 3.5$ , the S-strategists mainly belonged to Y, F, G, J, and  $L_0$  functional groups. Conversely, during the periods when the lake showed an anticipated breaking of the thermocline (B periods in Fig. 1), functional groups of S-strategists showed a progression of  $L_0 \rightarrow L_M \rightarrow M$ , as the underwater light availability decreased. This pattern was constantly repeated year by year, and the M functional group exclusively collected *Microcystis* spp. Thus, 2 main changes could be recorded in the reservoir during periods characterized by the summer break in the thermocline: disappearance of C-strategists, and a replacement of functional groups among S-strategists. Conversely, composition of R-strategists was almost constant throughout the study period.

**Table 1.** Main species and functional groups collecting S-strategists in the three periods (A, B, C) identified during the study.

Lake periods	Functional group	Main representatives	Notes
A and C periods: 1990–1992; 1994–1997; 2003 onward	G	<i>Pandorina morum</i> , <i>Eudorina elegans</i>	mainly 1991–1992
	J	<i>Pediastrum</i> s.l., <i>Coelastrum microporum</i> , <i>C. astroideum</i> , <i>Harriotina reticulata</i> , <i>Scenedesmus</i> spp., <i>Desmodemus</i> spp., <i>Crucigenia</i> spp., <i>Tetraedron</i> spp.	This group was also present in 1993 along with group F. They were replaced by L <sub>O</sub> in July, L <sub>M</sub> in August and M in late summer
	F	<i>Botryococcus brauni</i> , <i>B. terribilis</i> , <i>Pseudosphaerocystis lacustris</i> , <i>Oocystis lacustris</i> <i>Mucidosphaerium pulchellum</i> , <i>M. planctonicum</i> , <i>Micractinium pusillum</i> <i>Ankistrodesmus</i> spp.	
	Y	large <i>Cryptomonas</i> spp., small <i>Gymnodinium</i> spp.	
	L <sub>O</sub>	<i>Peridinium</i> spp., <i>Peridiniopsis</i> spp. <i>Ceratium hirundinella</i> , <i>C. furcoides</i> , <i>Merismopedia</i> sp., <i>M. tenuissima</i> , <i>Woronichinia naegeliana</i> <i>Snowella lacustris</i>	This functional group of S-strategists was also characteristic of C periods but with a lower number of representatives
B periods: 1993; 1998–2002	L <sub>O</sub>	<i>Ceratium hirundinella</i> , <i>C. furcoides</i>	
	L <sub>M</sub>	<i>Ceratium hirundinella</i> , <i>C. furcoides</i> , <i>Microcystis</i> spp.	
	M	<i>Microcystis aeruginosa</i>	

**Fig. 5.** Relative contribution to total biomass of the 3 survival strategies of phytoplankton (C, S, R) in Lake Arancio, Italy, during 1990–2005.

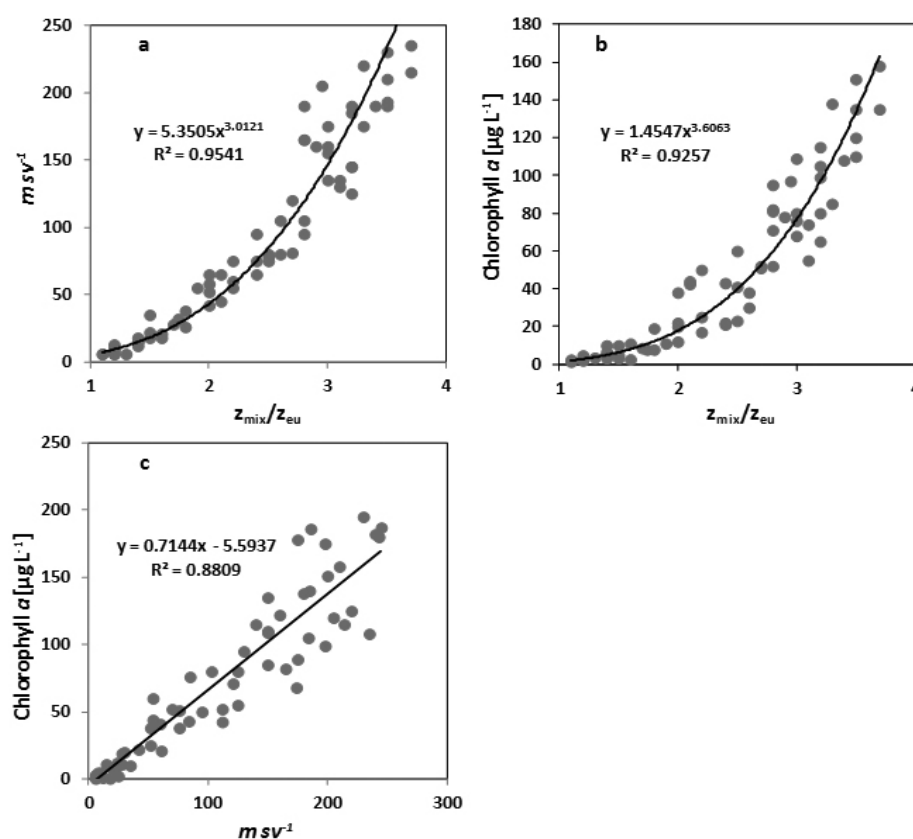
## Relationships between environmental variables and morphological descriptors

A significant positive relationship was found in Lake Arancio plotting  $m\text{ }sv^{-1}$  against  $z_{\text{mix}}/z_{\text{eu}}$  during 1990–1993 (Fig. 6a). Values of chlorophyll *a* measured in the same period also showed a positive relationship when plotted against  $z_{\text{mix}}/z_{\text{eu}}$  (Fig. 6b). These 2 relationships were similar, and a linear correlation was found when chlorophyll *a* was plotted against  $m\text{ }sv^{-1}$  (Fig. 6c). No correlations were found between  $m\text{ }sv^{-1}$  and chlorophyll *a* concentrations using data from 2001–2002.

## Discussion

### Hydrological and thermal patterns

Water level fluctuations occur naturally in almost all aquatic ecosystems (Zohary and Ostrovsky 2011), but they can interfere with the natural physical and chemical lake processes when their amplitude is large compared to the depth of the lake. Man-made lakes in the Mediterranean area are regulated ecosystems constructed to provide water for drinking and irrigation during the summer dry periods, which characterize Mediterranean climate. Thus, their levels are prone to decreases during



**Fig. 6.** Relationships between (a)  $z_{\text{mix}}/z_{\text{eu}}$  and  $m \text{ sv}^{-1}$ ; (b)  $z_{\text{mix}}/z_{\text{eu}}$  and chlorophyll *a* concentrations; and (c)  $m \text{ sv}^{-1}$  and chlorophyll *a* concentrations during 1990–1993.

summer when precipitation is scarce or absent and when water (over-)exploitation occurs to fulfill social and production requests. Over-exploitation of water may significantly alter the physical structure of lakes, disrupting stratification patterns (Rimmer et al. 2011).

Water level fluctuations are particularly large in Sicily, an island located in the middle of the Mediterranean Sea with a river network mainly formed of temporary streams (Naselli-Flores 2011). Several of these streams were dammed in the last 60–70 years to create man-made lakes and enhance water availability. The increased water availability encouraged agricultural expansion of water-demanding crops and established a water market that stimulated strong and often illegal economic interests (Giglioli and Swyngedouw 2008), further contributing to enhanced water level fluctuations.

Lake Arancio is potentially a warm monomictic lake, but agricultural water-demand can quickly reduce its depth and cause a midsummer break in the thermocline. In relation to its maximum annual filling during the investigation period, however, the lake could be considered a shallow waterbody circulating throughout the year; a warm monomictic lake with a stable summer stratifica-

tion; or something intermediate—a warm monomictic waterbody in spring and a fully circulating one in midsummer (Naselli-Flores 2003).

### Underwater light regime

Underwater light availability is a key factor regulating phytoplankton dynamics (Zohary et al. 2010), and thermocline breaks have consequences on the light regime of Lake Arancio. Following thermocline breaks, a sudden increase of  $z_{\text{mix}}/z_{\text{eu}}$  was observed, which contributed to turn this environment optically deep. The ratio  $z_{\text{mix}}/z_{\text{eu}}$  is a good indicator of light availability for phytoplankton because it directly indicates the proportion of time that fully entrained phytoplankton must spend at critical light intensities. A decreased availability in underwater light can be promoted either by a decrease of the euphotic depth or by an increase in the mixing depth. Both of these events occur commonly in Mediterranean reservoirs found in the upper part of the trophic range and have a direct influence on phytoplankton composition and growth (Naselli-Flores 1998). Assuming a constant respiration rate of 10% of maximum



photosynthetic rate, net growth of entrained phytoplankton cannot occur when  $z_{\text{mix}}/z_{\text{eu}}$  is  $>3$  (Reynolds 2006). Gas-vacuolated cyanobacteria such as *Microcystis* spp. may easily escape entrainment and thus prevail under these light conditions.

### Nutrient availability

Nutrients likely did not contribute to phytoplankton growth regulation in Lake Arancio. Diffuse sources from intensive agriculture in the watershed and untreated point sources have provided an almost unlimited nutrient source to phytoplankton. The lowest SRP concentrations ( $6\text{--}8\ \mu\text{g P L}^{-1}$ ) were always measured in spring, indicating an active exploitation by phytoplankton. The highest values ( $60\text{--}100\ \mu\text{g P L}^{-1}$ ) were recorded in summer (1990–1993) or winter (2001–2004). Nutrient loading from the catchment mainly occurred in the wet season (mid-autumn–early spring). Because the capacity to retain phosphorus is correlated to residence time, and the reservoir received the highest nutrient loads in winter when no significant water outflow occurred (residence time tended to infinity), the reservoir acted as a phosphorus sink (Straškraba et al. 1995) and its internal loading steadily increased. A similar pattern was observed in Lake Kinneret, the largest Mediterranean natural lake (Hambright et al. 2004). When the lake was first regulated to sustain anthropogenic water requests, its internal loading began to increase, promoting eutrophication processes.

The phosphorus pulses observed in summer, when precipitation was scarce or absent, mainly occurred from internal loading. The hypolimnetic environment of this productive ecosystem was characterized by the absence of oxygen and low pH values, which favored the remobilization of phosphorus from sediments (Naselli-Flores and Barone 2005). Nutrient-rich hypolimnetic waters then entered the epilimnion in 2 ways:

As the water was abstracted, the water level decreased and the thermocline was lowered. As a consequence, formerly hypolimnetic waters as well as part of the hypolimnetic bottom became part of the epilimnion (Naselli-Flores and Barone 2005).

A further connection between hypolimnetic and epilimnetic waters occurred during warm and calm summer days, when atelomictic events (daily stratifications) were frequently observed in periods A and B (Barone and Naselli-Flores 1994, Naselli-Flores 2003). Daily stratification promoted rapid oxygen consumption and lowered pH, which favored phosphorus release, which was then again available to sustain phytoplankton growth when circulation was reestablished in the evening.

### Morphology-based phytoplankton analysis

Among functional traits of phytoplankton (e.g., presence of flagella, ability to form colonies, ability to fix nitrogen, presence of vacuoles or gas vesicles, typology of cell wall, and typology of storage substances), morphological features of shape and size represent the starting point to fully understand the relationships between this group of organisms and its environment (Naselli-Flores and Barone 2011).

Predicting phytoplankton dynamics based on observed initial conditions can be a difficult task (Bauer et al. 2013). In Mediterranean reservoirs, however, seasonal and annual changes in the physical environment trigger seasonally predictable autogenic dynamics of phytoplankton (e.g., Hoyer et al. 2009, Padisák et al. 2010, Rigosi and Rueda 2012), as observed in the present study by analyzing the dynamics followed by the C-S-R survival strategies over a long term.

Analysis of the morphological features of dominant phytoplankton in Lake Arancio allowed us to group them in the 3 main survival strategies described by Reynolds (2006 and literature therein). These broad groups followed consistent dynamics in years when the lake could be classified as shallow or deep. In both shallow and deep periods, a C→S→R succession was observed, which largely corresponds to the theoretical autogenic successional pattern of freshwater phytoplankton. C-strategists, invasive species investing in rapid growth and requiring high availability of resources (light and nutrients), were dominant in winter and early spring; followed by S-strategists, more acquisitive species investing in nutrient conservation, in late spring to early summer; followed by R-strategists, more acclimating species investing in efficient light conversion, which replaced S-strategists in late summer and autumn.

These patterns reflect the availability of light and nutrients in Lake Arancio. In winter and early spring nutrients were abundant and light availability was relatively high. As phytoplankton growth increased, nutrients were consumed, but light was not limiting because of the shallowness of the waterbody or because of thermal stratification. Light availability in late-summer and autumn was reduced by continued growth of phytoplankton, initially fueled by hypolimnetic nutrients entering the epilimnion as water level decreased. Later, the beginning of the wet season reestablished nutrient income from the watershed.

During years when summer water exploitation was sufficient to interfere with the thermal stability of the lake, the water column was characterized by a sharp decrease in underwater light availability due to the deepening of the mixing depth, and by additional nutrient inputs from the

sediments driven by atelomixis in midsummer. The high values of  $z_{\text{mix}}/z_{\text{eu}}$  selected phytoplankton from the species assemblage that were able to completely escape entrainment in water motion, represented exclusively by buoyant cyanobacteria belonging to the genus *Microcystis* (Naselli-Flores 2003, Naselli-Flores and Barone 2003, 2005, 2007). Typically, S-strategists *Microcystis* spp., which were present but never dominant in the assemblage, became the main actors in phytoplankton dynamics in Lake Arancio, especially during 1998–2002.

*Microcystis* dominance was further enhanced by the lowering water level in summer, causing a progressive exposure of the lake bottom where large inocula overwinter (Naselli-Flores 2003). Coupled with the effect of waves along the shore, this drying effect allowed the resuspension of colonies, likely favoring a progressive recruitment of *Microcystis* from the sediment (Verspagen et al. 2004). Once dominance was established, more or less subtle changes in the environmental conditions, such as wind-induced mixing or water inputs from the catchment and the subsequent dilution of colonies, had no effect, especially when the environment was already overrun with *Microcystis* (Naselli-Flores et al. 2003). Conversely, nutrient inputs further sustained *Microcystis* growth throughout summer, causing concern in the lake Management Board because of toxin production (Naselli-Flores et al. 2007) and sustained a positive feedback that allowed *Microcystis* to persist in the optically deep Lake Arancio (Naselli-Flores and Barone 2003). Moreover, as also noted by O'Brien et al. (2004), *Microcystis* dominance was favored by modifications in the morphology of colony size and shape.

The shift from an underwater environment occasionally showing unsuitable light characteristics to one almost permanently poor in light had several influences on phytoplankton survival strategies. The pool of species able to survive in a stressed environment becomes progressively smaller as stress increases (Naselli Flores et al. 2003). During these periods of reduced underwater light availability, phytoplankton in Lake Arancio were mainly represented by K-selected species from the S and R strategic groups. In particular, S-strategists mainly belonged to the M functional group, whereas R strategists belonged to the P functional group. Both of these groups collect stress-adapted organisms, and the r-selected C-strategists were likely unable to cope with the harsh environmental conditions and disappeared.

The 2003 Lake Arancio Management Board modification to refill the lake using water from another reservoir contributed to reestablishment of the C→S→R successional pattern (Naselli-Flores and Barone 2005). This succession allowed thermal stratification to maintain throughout the summer, increased the underwater light

availability, and decreased the input of nutrients from the hypolimnion. The better-illuminated water column thus favored the growth of green algae, which have growth rates much higher than *Microcystis* spp. (Lüring et al. 2013) and were thus able to outcompete cyanobacteria, greatly reducing their abundances.

### Trophic state and morphological descriptors

The dependence of phytoplankton-specific rates of growth at subsaturating light intensities was experimentally demonstrated to be a correlative of algal unit morphology (Reynolds 1997). The value of  $m \text{ sv}^{-1}$  is least for the sphere shape and is equal to 6 independently by any increase in diameter. The more the sphere is deformed in ellipsoidal or even rod-like or filamentous shapes, the higher the value of this shape descriptor.

The evolutionary pressure promoting the relationship between growth rates at markedly subsaturating light and the shape descriptor is likely due to the better exposure of chloroplasts to light in elongated, needle-shaped phytoplankters (e.g., *Closterium aciculare*) compared to spherical or ellipsoidal ones with the same volume (Naselli-Flores and Barone 2011). In Lake Arancio during 1990–1993, the existence of a nonlinear, significant relationship was found between the descriptor used for underwater light regime,  $z_{\text{mix}}/z_{\text{eu}}$ , and  $m \text{ sv}^{-1}$ . The power of this relationship was similar to that found when  $z_{\text{mix}}/z_{\text{eu}}$  was plotted against chlorophyll *a* concentrations, and a significant linear correlation was found when plotting  $m \text{ sv}^{-1}$  against chlorophyll *a* concentrations. Although relationships between  $z_{\text{mix}}/z_{\text{eu}}$  and  $m \text{ sv}^{-1}$  were also found when  $z_{\text{mix}}/z_{\text{eu}}$  was  $>3.5$  (Naselli-Flores and Barone 2007), a significant correlation between  $m \text{ sv}^{-1}$  and chlorophyll *a* concentrations was not found during those periods characterized by high  $z_{\text{mix}}/z_{\text{eu}}$  (2001–2002), probably because of the physiological limits to chlorophyll production and to the prolonged dominance of large, buoyant *Microcystis* colonies.

### Conclusions

Phytoplankton integrate physical, chemical, and biological parameters of freshwater ecosystems, and long-term datasets and/or the comparison of morphological structure of phytoplankton in different periods can be reliable indicators of environmental changes and trends (Barone et al. 2010). The morphological analysis of phytoplankton conducted in Lake Arancio provided a reliable summary of the long-term ecological variability that characterizes this man-made lake. An accurate interpretation of the dominant survival strategies in phytoplankton assemblages can be a powerful tool to clarify

ecosystem functioning and to assess water quality and trophic state. Morphological descriptors such as surface, volume, and their ratio, as well as maximal linear dimension and its product with  $sv^{-1}$ , can be powerful predictors of physiological performances of phytoplankton and offer a valid interpretation of environmental conditions. In particular, this method, which does not require taxonomical skills, can be a useful tool for monitoring purposes and is more reliable than species composition, especially when identification of phytoplankton is conducted by nonexperts.

## Acknowledgements

I am deeply indebted to Judit Padisák and Vera Istvánovics for their helpful comments and suggestions on a previous draft of the manuscript. A grant from the University of Palermo (2012-ATE-0148) is also acknowledged.

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